UGA Archaeal Collaboration Study Protocol

Thank you for participating in the first Archaeal Collaboration Study, hosted by the University of Georgia iGEM Team! Below you will find a protocol to follow for quantifying the fluorescence of your samples. Please do not hesitate to contact us should you have any questions or encounter any ambiguities.

Important: As soon as you receive your samples in the mail, place them in a freezer (-80ºC) in order to reduce potential protein damages that may incur from freezing and thawing.

We recommend that you place your samples in the freezer and take time to read over our protocol as well as prepare your plan of action.

You have been given 30 total samples, (i.e., triplicate cell extracts obtained from 10 colonies, and please stay in accordance with our labels throughout your experiment.)

Each sample contains 10µL of cell extracts. The buffer used is 25mM PIPES pH 6.8. The PIPES should act as a negative control in your experiment. If PIPES is not available, a buffer with the same pH may be sufficient.

1. Remove samples from the freezer and place in a shaker at 30^{O}C for overnight incubation. (This step is ***necessary*** because our cells are grown anaerobically but the fluorophore of mCherry fluorescent protein requires sufficient oxygen exposure for maturation.)
2. Using "8B" determine the linear range for your measurement device.
    a. We recorded the highest fluorescence from sample "8B," so if this is found to be in the linear range, then all the other samples will be as well.
    b. Our device required a 200µL sample, so we found that a 100X dilution (i.e., 2µL cell extracts re-suspended in 198µL PIPES buffer) was within the linear range.
    c. Determining the linear range can be done with serial dilutions (e.g., 10, 10^2, 10^3, 10^4 and 10^5 dilutions).
3. Our measurement device is a BioTek Synergy HT plate reader (96 well) and we use the Gen5 software.
    a. Excitation wavelength (nm): 590/20
    b. Emission wavelength (nm): 645/40
    c. Scaled* to High Wells
    d. Setpoint Temperature: 25^{O}C
4. Your measurement device and measurement protocol may vary from ours and other teams, so please update our form with your specific parameters. Email Steven Kodish at skodish@uga.edu, Rebecca Anne Buchanan beccab@uga.edu, and Akshay Chandora at akshaych@uga.edu upon receiving this shipment for the link to the registration form and data form or for any additional questions.

*Our values are all relative to each other (i.e., X is 20% more fluorescent than Y) so that all data can be correlated regardless of measurement device.